Preventive Effect of Treatment of Bacillus Subtilis DB-9011 on Dogs of which Cellular Immune Function is Decreased by Surgical Operation

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Purpose:

There were many reports that the cellular immune function was used to be decreased after surgical operation. As to this decrease of cellular immune function after surgical operation, there is observed and reported that the decreased function accelerate metastasis in cases of postoperative infection diseases and neoplasma.

There were reported that Bacillus Subtilis DB-9011 had effects on activation of cellular immune function in mouse, swine and cattles. Therefore, we have tried to study whether the treatment of Bacillus Subtilis DB-9011 which is lyophillized to dogs before operation, experimental gastrotomy, could be able to prevent the cellular immune function by using luminol dependent chemical luminescence method (=CL method).

Materials and methods:

1. Experimental materials;

(1)Animals; 23 Beagles and mongrels which are checked to be health clinically were subdivided to 2 groups; 18 control dogs without treatment of DB-9011 and 5 treatment dogs with DB-9011.

(2)Drugs; Bacillus Subtilis DB-9011 which is lyophillized (2x108/kg) supplied by AHC Inc.

2. Experimental methods;

(1)Treatment; In Treatment group, dogs were treated orally with DB-9011 successive days during 7 to 2 days before operation and 2 to 14 days after operation.

(2)Anesthesia and operation methods; Anesthesia; Dogs were treated with atropine sulfate (0.04 mg/kg) sc., as pre-treatment, and 15 min. after pretreatment, treated with chlorpromazine hydrochloride (0.5 mg/kg) im., and introduced with treatment of thiopental sodium (12.5 mg/kg) iv., and kept with aspiration of 1.5 to 2.0% of halothane. Operation; under these conditions, experimental gastrotomy was conducted.

(3)Blood test and CL methods; Collections of blood from cervical vein were conducted at the days before treatment of DB-9011, the day before gastrotomy, immediately after operation, 1 to 3 days, 5 days, 7 days, 10 days and 14 days after operation. Blood was tested at the following items.

① General Blood tests; 1 ml of blood with EDTA was used to measure red blood cell count, leukocyte count, packed-cell volume (PCV) and differential leukocyte count.

② measure the activity of neutrophil of whole blood, and the remaining blood was used for isolation of mononuclear cells.

Mononuclear cells were collected by specific gravity centrifugaion method, and were flotated in dish. After incubation during 1 hr., macrophages were collected as adherent cells. These methods were used to measure the activity of macrophages. Peripheral blood lymphocytes (PBL) were also collected as adherent cells, and this was used to measure the activity of NK cells.

PBL was prepared into culture solution of RPMI-1640 added by 10 % of fetal bovine serum as 4x106/ ml, and macrophage was prepared into the same culture solution as 1x106/ml.

③ Measure of CL activity of some kinds of immunocyte; KAC-2 was added as stimulator to whole blood and macrophages, and CL-1 cells from cynothymia type of lymhangiosarcoma.

Vertical line of graph shows number or activity, and horizontal line shows days after administration. 0 of successive days treatments in horizontal line means pre-treatment of DB-9011, and this value is expressed as 100 %. −1 means the day before operation, and this value is expressed as 100 % in non-treatment group.

In variation of the activity of neutrophil after successive treatments of DB-9011, the peak level was reached at the day 5 (Fig. 1).

In the activities of macrophages and NK cells, the peak value were also showed at day 5 (Fig. 2).

Leukocyte counts of treatment group were considerably increased by comparing to non-treatment group over the days after operation. Futermore, neutrophile counts were significantly increased to be proved by differential leukocyte count, therefore, the increase of leukocyte counts was thought to be made by the incresed of nuutrophile counts (Fig.3).

Neutrophile activity had showed a little decrease immediately after operation in treatment group, but the value of treatment group was 2.5 hold higher than the value of non-treatment group at the day 7 when the value of non-treatment group was decreased less than the value of pre-operation time (Fig.4).

In the activity of macrophages of non-treatment group, the value was remarkably decreased at the day 1 after operation, and those were decreased at day 5 and day 10, but the value of treatment group was increased to 3.5-hold at day 1 and the high value remained until day 14 after operation (Fig.5).

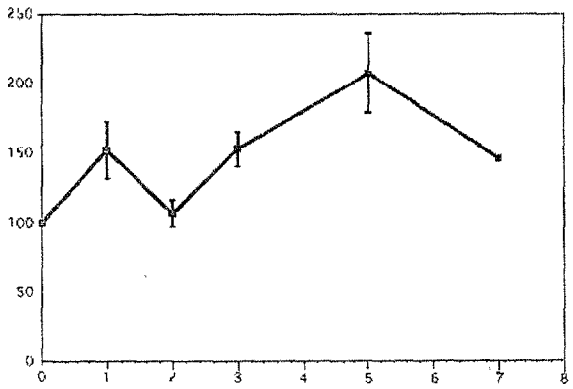
The activity of NK cells had also showed the similar pattern to the activity of macrophage. In the treatment group, the value of dya 1 was decreased less than the value of immediately after operation, but it was not so decreased as to the value of non-treatment group, and it remained high level later on (Fig.6).

Discussion:

The values of neutrophile count, macrophages activity and NK cells were most increased by the successive treatments of DB-9011 at day 5.

By treatments of DB-9011, the decrease of neutrophie count showed in non-treatment group at day 7 was prevented in treatment group. Furthermore, decreased activity of macrophages and NK cells showed in non-treatment group at day 1 was also prevented by treatments of DB-9011.

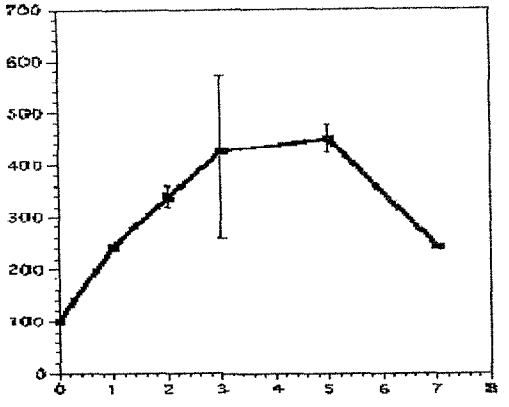
Therefore, it is strongly suggested that the treatment of DB-9011 before operation will be useful for prevention of the decrease of cellular immune function.



Neutrophil Activity (%)

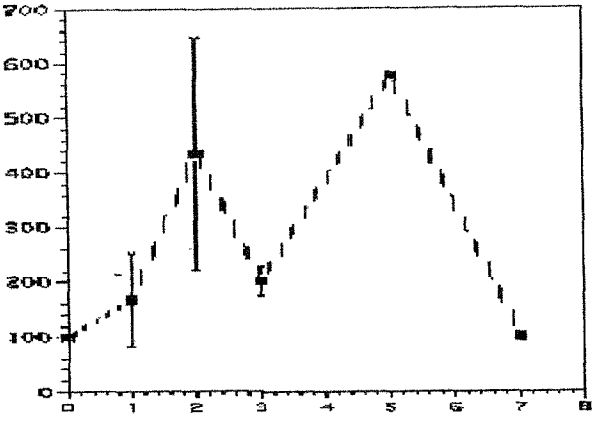
Days of Administration

Macrophage Activity (%)



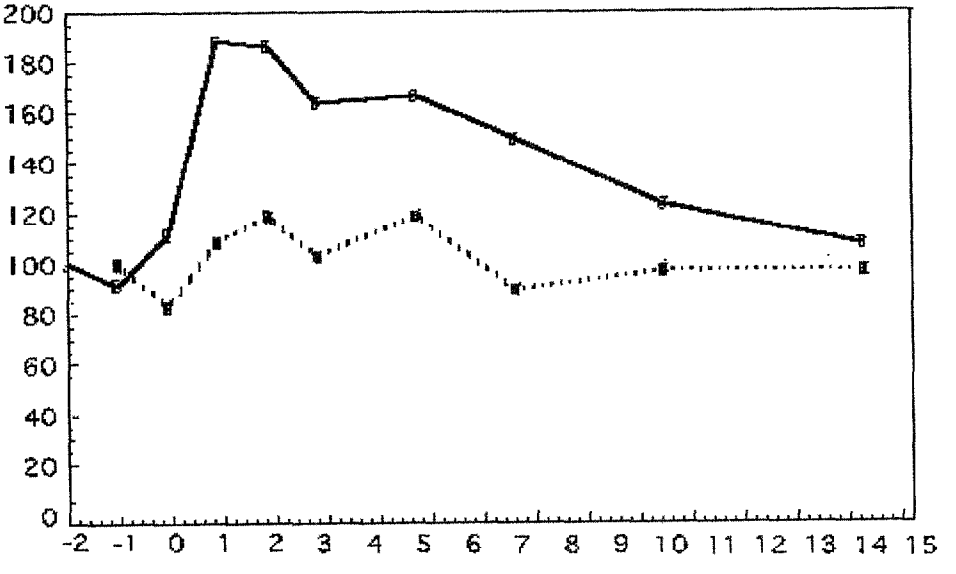
Days of Administration

NK Cell Activity (%)



Days of Administration

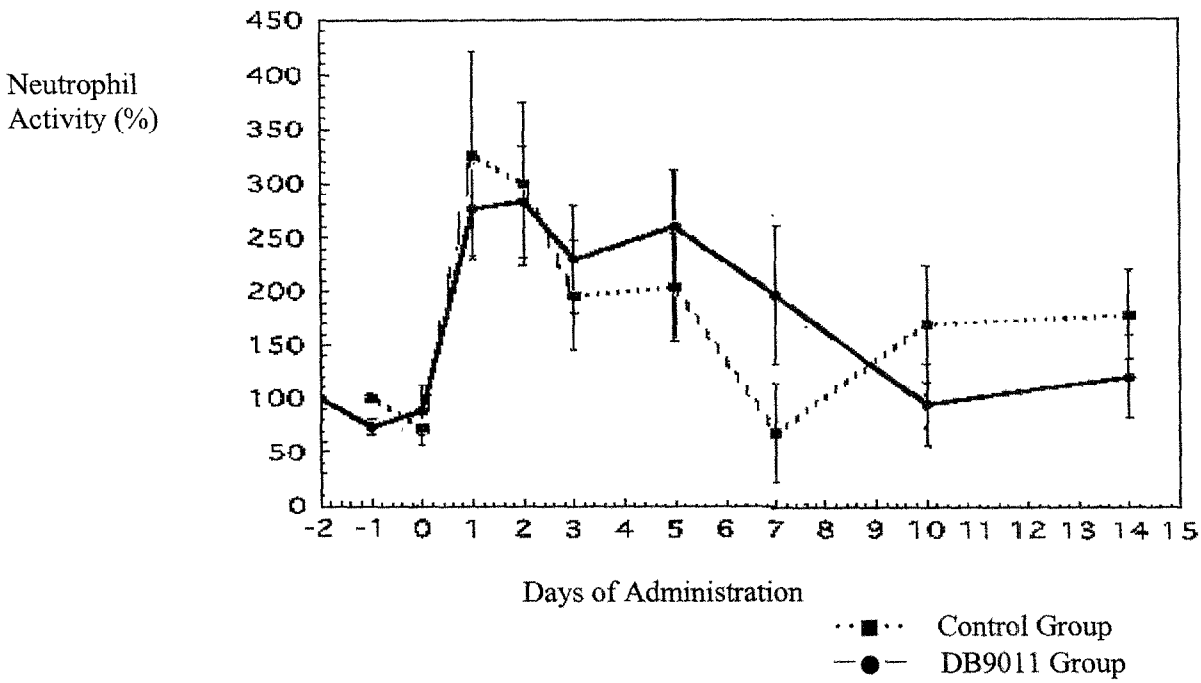
Amount of White Blood Cell (%)



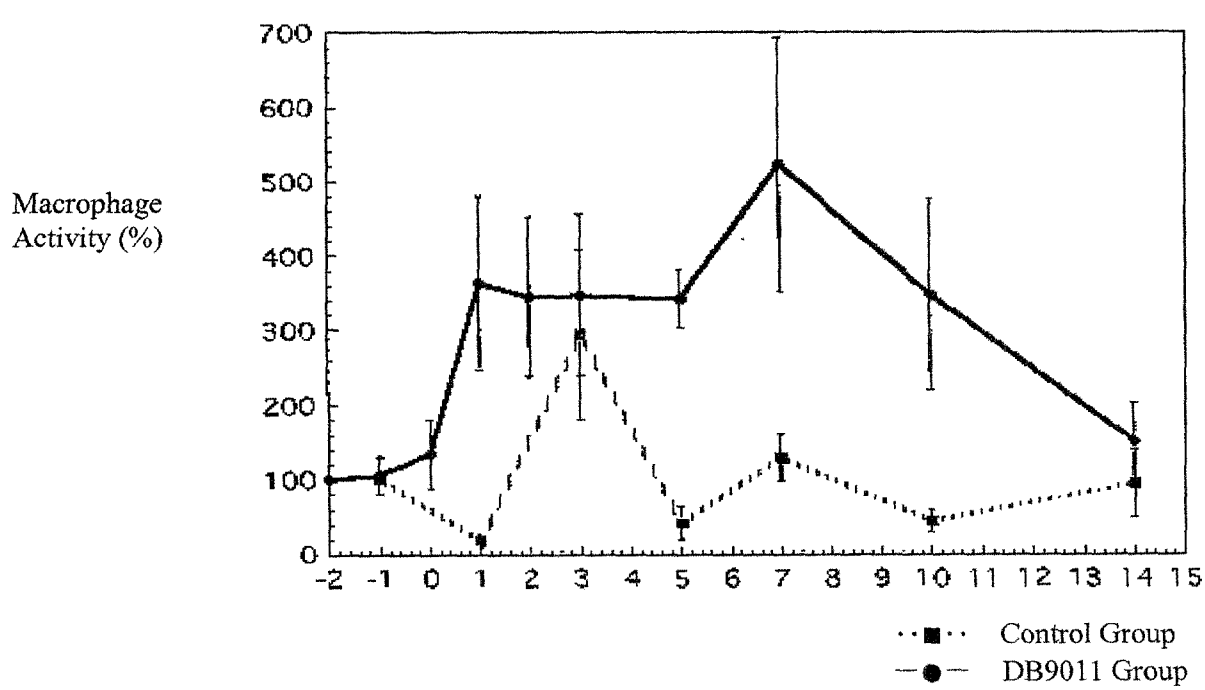
Movement of White Blood Cell by DB9011 after Surgery

—□— DB9011 Group ··■·· Contrlol Group

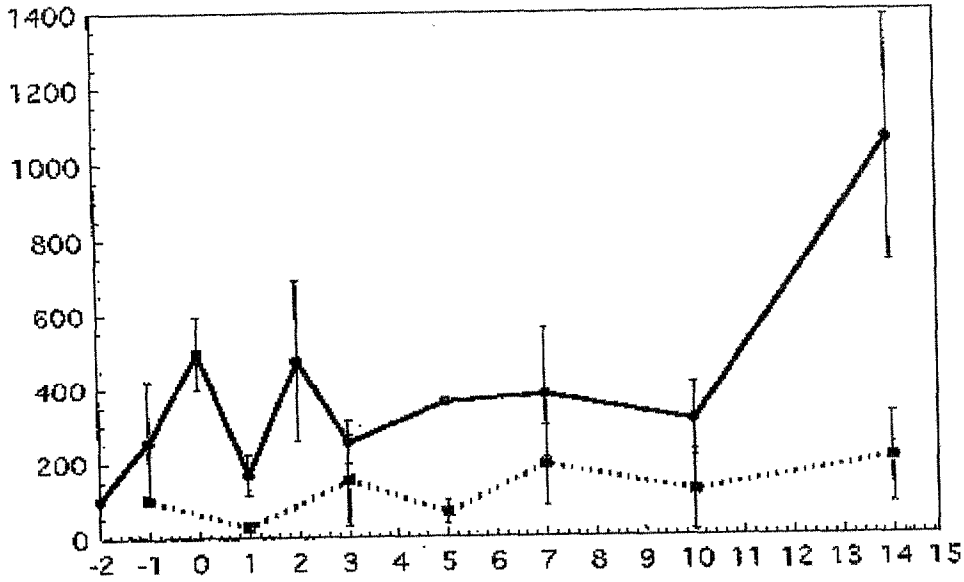
Movement of Neutrophil Activity by DB9011 after Sugery



Movement of Macrophage Activity by DB9011 after Surgery



Movement of NK Cell Activity by DB9011 after Surgery



Days of Administration

··■·· Control Group

—●— DB9011 Group